REMARKS

In order to more particularly claim the subject matter which applicants regard as their invention, claims 13 and 25-28 have been canceled, without prejudice, and claims 1-2 and 4-6 have been amended. Claims 1-2 and 4-11 are currently pending.

Specifically, applicants have amended claim 1 to recite a method for improving heart function in a patient having cardiac scar tissue by administration of mesenchymal stem cells to said cardiac scar tissue. Support for the amendment may be found throughout the specification as filed. See, for example, page 2, line 19 to page 3, line 3. Applicants have amended claims 2, 4 and 6 to change their dependency and amended claims 2, 4, 5 and 6 to clarify the language. The changes in dependency serve to broaden the claims. The clarifications of language are not made for reasons related to patentability, but merely to make explicit what was previously implicit. Support for the amendments may be found throughout the specification as filed. No new matter has been added.

Applicants acknowledge with appreciation the Examiner's withdrawal of the previous claim rejections under 35 U.S.C. §102(b). The remaining claim rejections will be discussed in detail below.

Claims 1 and 25 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite by the phrases "repairing" and "repairs." Claim 25 has been canceled and amended claim 1 no longer uses the terms "repair" or "repairing." Thus, the §112, second paragraph rejection is moot.

Claims 1, 2, 4-11, 13 and 25-28 stand rejected under 35 U.S.C. § 112, first paragraph. Specifically, the Examiner asserts that the limitations "repairing scarred

myocardial tissue" and "scar tissue repairs" have "no support in the as-filed specification" because, while "there is exemplified disclosure directed to improvement of cardiac function ... it is uncertain what is the difference between cardiac function improvement ... and 'repairs' or 'repairing'...."

As explained above, amended claim 1 no longer recites "repair" or "repairing" and those terms also do not appear in claims 2 or 4-11. Thus, all pending claims meet the requirements of 35 U.S.C. § 112, first paragraph.

Claims 1, 2, 4-11, 13 and 25-28 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over U.S. 5,602,301 ("the '301 patent"), Robinson et al., "Implantation of Skeletal Myoblast-Derived Cells", in Cellular Cardiomyoplasty: Myocardial Repair with Cell Implantation, ed: Kao and Chiu, 1997 ("Robinson"), Murry et al., *J. Clin. Invest.* 98:2512-23 (1996) ("Murry"), and/or WO 99/03973, taken with Wakitani et al., *Muscle & Nerve*, 18:1417-26 (1995) ("Wakitani") and U.S. 5,736,396 ("the '396 patent").

Specifically, the Examiner contends that the '301 patent, Robinson and Murry teach methods for treating myocardial disorders by administering cells of the myogenic lineage into myocardial tissues, that WO 99/03973 teaches methods for treating myocardial disorders by administering MSC's into myocardial tissues, that Wakitani teaches treating MSCs with 5-azacytidine and teaches using MSCs for the purpose of transplantation, and that the '396 patent teaches the use of MSCs treated with 5-azacytidine for treating tissue disorders. The Examiner concludes that it would have been obvious to the ordinarily skilled artisan to substitute the cell compositions of Wakitani and the '396 patent for the myoblast cells of the '301 patent, Robinson and Murry,

because "the prior art teaches treating myocardial disorders by administering into myocardial tissues, including damaged or injured myocardial tissues ... cells belonging to [the] myogenic lineage" and because "MSCs are advantageous over myoblast compositions which require large muscle biopsy." The Examiner further contends that applicants' arguments filed April 16, 2002 are not persuasive because the cited references "encompass transplantation into myocardial scar tissue" and that the models used in the cited art are "the same model" as is used by applicants." Applicants traverse.

It is well recognized in the art that acute myocardial injury is followed by identifiable stages of wound healing during which the cellular environment within the injured heart changes dramatically. See, for example, Murray at page 2513, first column, which reports that in the animal model used therein, "the cellular patterns of coagulation necrosis, inflammation and phagocytosis, granulation tissue formation, and scarring after freeze-thaw injury are indistinguishable from myocardial infarction...."

More particularly, it is known from histological analyses that immediately after an acute myocardial injury, the myocardium becomes fragmented and necrotic. Tissue necrosis is followed by inflammation. Specifically, within about a week, most of the necrosed cardiomyocytes have disappeared and a predominantly mononuclear inflammatory infiltrate is present. By about two weeks after injury the inflammatory infiltrate disappears and the fibroblasts and collagen that comprise scar tissue become evident. At four and eight weeks after injury, the tissue is fibrotic and no longer contains cardiac muscle cells or lymphocytes. Instead, it is composed of connective tissue cells, such as fibroblasts, and non-cellular components, such as collagen and fibronectin.

Cardiac scar tissue is non-contractile, and is believed to be an inert tissue having a limited blood supply.

As will become evident from the discussion below, the art cited by the Examiner refers to cellular implantation into normal heart tissue, acutely damaged (i.e., necrotic) heart tissue, or granulation tissue within the heart. None of the cited art refers to implantation of mesenchymal stem cells into cardiac *scar* tissue. Furthermore, given that mesenchymal stem cells are pluripotent cells which are believed to be highly sensitive to their surrounding environment, none of the cited art provides any reasonable expectation that mesenchymal stem cells could improve heart function when implanted into the inhospitable environment of cardiac scar tissue.

Moreover, applicants respectfully disagree that the animal models they have utilized are the "same models" as reported in the art. One of the major differences results from the timing of administration of the cells. Specifically, applicants direct the Examiner to the specification at page 9, lines 4-9, which states that in applicant's rat cryoinjury model, the mesenchymal stem cells were implanted *three weeks* after injury (see also page 17, line 23 to page 18, line 2, page 20 lines 4-5). Similarly, page 25 lines 10-13 demonstrate that applicants' swine infarct model involved administration of cells *four weeks* after coronary artery ligation. Applicants submit that these models differ significantly from the models used in the cited art because in applicants' models, there was sufficient time after injury and before administration of cells that necrosis and inflammation had subsided and scar tissue had formed. In contrast, as will be discussed below, the cited art refers to administration of cells to the normal heart or to the acutely injured heart *prior to* the formation of cardiac scar tissue.

The '301 patent refers to the formation of myocardial grafts by implantation of skeletal myoblasts or cardiomyocytes into normal myocardial tissue of a mammal. It makes no reference to mesenchymal stem cells and makes no reference to implantation of cells into cardiac scar tissue.

Robinson reviews the art with respect to the fate of immortalized myoblasts implanted into the heart via various routes of administration. It does not teach or suggest implantation of mesenchymal stem cells into cardiac *scar* tissue, nor does it suggest that mesenchymal stem cells would be capable of surviving after implantation into cardiac scar tissue much less improve heart function.

Murry describes implantation of skeletal myoblasts into the heart during the inflammatory phase of wound healing – i.e., before the formation of scar tissue. Specifically, Murry reports on page 2513 that when the cells were implanted at one week after acute cyroinjury, "most of the necrotic myocardium had been replaced by granulation tissue, but scar formation had not yet begun."

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WO 99/03973, Wakitani and the '396 patent all refer to mesenchymal stem cells, but none suggest they would be capable of improving heart function after implantation into cardiac scar tissue. Specifically, WO 99/03973 (like its United States counterpart U.S. 6,387,369) refers to implantation of mesenchymal stem cells into "damaged" myocardium but never discusses the survival potential of mesenchymal stem cells implanted into *scar* tissue. Moreover, the examples are limited to implantation into normal cardiac muscle and the document as a whole teaches that "environmental signals ... act in concert with mechanical and electrical signaling *in vivo* to lead to cardiac differentiation" (see page 8, lines 28-30). Given that scar tissue would likely be devoid

of mechanical or electrical signaling, applicants' discovery that mesenchymal stem cells implanted into scar tissue can actually survive and improve cardiac function would not have been expected in view of WO 99/03973.

Wakitani reports that mesenchymal stem cells exposed *in vitro* to the compound 5-azacytidine appear to differentiate into myogenic and adipocytic phenotypes. It does not teach or suggest that mesenchymal stem cells could survive or improve heart function after implantation into cardiac scar tissue, either with or without prior exposure to a differentiating agent. To the contrary, Wakitani reports on page 1425 that "[t]he generalization seems to be that the local implantation site would provide the cuing environment to drive the MSCs down the proper phenotypic pathway." Again, given the inhospitable environment provided by cardiac scar tissue, Wakitani provides no reasonable expectation that MSCs implanted into cardiac scar tissue would improve heart function.

burden

The '396 patent refers to methods for *in vitro* or *ex vivo* lineage directed induction of mesenchymal stem cells. Like WO 99/03973, it reports:

> "Mesenchymal stem cells (MSCs) are the formative pluripotent blast or embryonic-like cells found in bone marrow, blood, dermis, and periosteum that are capable of differentiating into specific types of mesenchymal or connective tissues including adipose, osseous, cartilaginous, elastic, muscular, and fibrous connective tissues. The specific differentiation pathway which these cells enter depends upon various influences from mechanical influences and/or endogenous bioactive factors, such as growth factors, cytokines, and/or local microenvironmental conditions established by host tissues."

Nowhere does the '396 patent teach or suggest that mesenchymal stem cells, whether pre-differentiated or undifferentiated, could survive and improve heart function when implanted into cardiac scar tissue.

In summary, none of the cited art, either alone or in combination, teaches or suggests that implantation of mesenchymal stem cell into cardiac *scar* tissue would result in improvement of heart function. To the contrary, the teachings of the art, taken together, would more readily lead to the expectation that implantation of a mesenchymal stem cell into cardiac scar tissue would not improve heart function because it would induce only the formation of additional scar tissue.

In view of the above, applicants request that the Examiner withdraw the rejections under 35 U.S.C. § 102(b) and pass the application to issue.

If it is believed that a discussion of these or other issues would be beneficial, the Examiner is invited to contact applicants' attorney at the numbers listed below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

p.2, line 15

1. (Twice Amended) A method for [repairing scarred myocardial] improving heart function in a patient having cardiac scar tissue, said method comprising administering to [myocardial] said cardiac scar tissue a cellular suspension containing mesenchymal stem cells, wherein [administration of] said administered cells survive in [to] said [myocardial] cardiac scar tissue [repairs said scarred myocardial tissue] and improve heart function in said patient. p.7 figure p. 6. last para.

2. (Twice Amended) The method of claim [25] 1, wherein said [at least one] mesenchymal stem [cell has] cells have been induced to differentiate into [a] cardiomyogenic cells prior to administration.

4. (Twice Amended) The method of claim [2] 1, wherein said mesenchymal stem cells have been cultured for at least 7 days prior to administration.

5. (Twice Amended) The method of claim 2, wherein said mesenchymal stem cells have been induced to differentiate by co-culture [cocultured] with cardiomyocytes.

6. (Once Amended) The method of [claim 1] any one of claims 1-2, 4-5 and 7-11, wherein said mesenchymal stem cells are autologous to the patient being treated.